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Immunotherapy of Crohn's disease

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ALTHOUGH **the initiating events of Crohn's disease are unknown, models of experimental colitis have provided new insights in the immunologically mediated pathways of mucosal inflammation. In Crohn's disease activated mucosal T lymphocytes produce pro-inflammatory cytokines within the mucosal compartment. With this understanding, there has been a shift in past years from the use of unspecific anti-inflammatory agents (corticosteroids, aminosalicylates) to the use of immunomodulatory drugs (azathioprine, methotrexate). Moreover, novel strategies have been designed for specific targets in Crohn's disease, in particular T lymphocytes and cytokines. In an open label study treatment of steroid-refractory Crohn's disease with anti- CD4+ antibodies was well tolerated and showed clinical benefit. However, a sustained depletion of the CD4+ cells precluded further clinical trials. In controlled clinical studies, anti-tumour necrosis factor (TNF-α) antibodies induced complete remissions and few side effects were observed. One study suggested efficacy in active Crohn's disease of recombinant interleukin-10. Long term treatment studies will have to answer questions about the indications for use, benefit and toxicity. Altogether, these results hold promise for future management of Crohn's disease, where disease-modifying interventions and strategies that effectively maintain disease remission will play a key role.**

Key words: Crohn's disease, medical treatment, new strategies, anti-CD4+ antibodies, anti-TNF-α antibodies, recombinant IL-10

Introduction

Crohn's disease is a chronic inflammation of the gastrointestinal tract, characterised by relapses and periods of disease remission. As the aetiology of this disease is unknown, medical treatment is symptomatical and aimed at surppressing the inflammatory response. To date, corticosteroids remain the mainstay treatment of active Crohn's disease, resulting in a rapid initial reduction of symptoms in approximately 70% of patients.[1] Unfortunately, many patients either become steroid-dependent, steroid resistant or suffer from side effects. An alternative therapeutic option is provided by immunomodulatory drugs such as azothioprine, methotrexate and cyclosporine.[2] The use of these agents is limited by their low efficacy, inadequate selectivity and substantial short and long term toxicity.

Recently, specific mediators of the immune response have been identified. In Crohn's disease there seems to be an enhanced mucosal T cell activation. Here, we briefly review several new immunomodulatory agents designed for specific targets, in particular T lymphocytes and cytokines, which have been studied in experimental and clinical inflammatory bowel disease.

Lessons from Colitis Models

Various experimental animal models of inflammatory bowel disease have partially unravelled the complex mucosal network of cytokine interactions.[4,5] The main conclusions from these studies can be summarised as follows: firstly, different subpopulations of T-cells within the CD4 positive (CD4+) compartment have a pivotal role in either initiation or control of the immune mediated mucosal inflammation. This paradigm is supported by observations made in T-cell mediated models of inflammatory bowel disease. IL-2 deficient ($IL\text{-}2^{null}$) mice crossed with β_2m^{null} mice, that lack functional CD8+ cells develop a spontaneous colitis.[6] Transfer of $CD45RB^{high}$ CD4+ T-cells (considered to be a Th1 precursor population) from normal mice to SCID mice (that lack T- and B-cells) resulted in a severe colitis, suggesting a causative role for this CD4+ subset.[7] The most efficient mean to prevent this intestinal inflammation was to co-transfer

CD45RB^{low} CD4+ T-cells (i.e., the regulatory T lymphocyte population).[8,9] Hence, lack of specific anti-inflammatory T-cells may lead to uncontrolled activation within the CD4+ compartment. Secondly, the importance of anti-inflammatory mechanisms is exemplified by a mouse model that has a targeted disruption in the IL-10 gene (IL-10 KO mouse). These mice develop a severe colitis with increased local levels of pro-inflammatory cytokines.[10] Thirdly, in the IL-10 KO mouse and in the CD45RB^{high} transfer model colitis does not occur in germ free animals. Therefore, the normal intestinal flora (or their antigens) are necessary for activation of the uncontrolled immune response in the mouse models mentioned. Finally, increased production of pro-inflammatory cytokines, including tumour necrosis factor-α (TNF-α) and interferon γ (IFNγ), is an important finding in experimental models where colitis is caused by chemical irritation of the intestinal mucosa by hapten induced T cell activation (e.g. TNBS model) or by T cell transfer.[11,12]

Collectively, these data seem to indicate that the normal mucosal immune response is strictly regulated and actively suppressed. An ill-controlled, antigen-dependent (CD4+) T lymphocyte activation will result in a high production of pro-inflammatory cytokines within the mucosal compartment and in inflammatory bowel disease.

Interestingly, the (repeated) administration of TNF-α, IL-12 and IFNγ neutralising antibodies and recombinant IL-10 resulted in amelioration of mucosal inflammation in several models of T cell dependent inflammation.[13,14,15]

Analogous to the findings in mouse models, in patients with Crohn's disease the mucosal production of various pro-inflammatory cytokines is increased, most likely as a consequence of chronic CD4+ T-cell activation.[16] The aim of new clinical interventions in Crohn's disease is either to decrease the activity of CD4+ cells, to neutralise pro-inflammatory cytokines such as TNF-α, or to increase anti-inflammatory cytokines that inhibit Th1 differentiation such as IL-10.[17,18,19]

Interference with T-cell Function

Two different ways of influencing T-cell function in inflammatory bowel disease have been investigated. First, a rather unspecific immunosuppressive approach using anti-CD4+ monoclonal antibodies was investigated in patients with Crohn's disease in order to assess safety and potential efficacy. The CD4 molecule is necessary for T-cell signal transduction following presentation of antigen by MHC class II molecules. In an uncontrolled pilot study, 12 patients with steroid refractory Crohn's disease were treated with a mouse-human chimeric antibody (cM-T412). This antibody depletes the number of circulating CD4+ cells, and interferes with CD4-dependent activation of T-cells. After seven consecutive infusions of 10, 30 or 100 mg daily only minor side effects, such as a febrile reaction after the first infusion, were experienced. Treatment resulted in a significant dose dependent reduction of the CDAI (10 weeks 24% and 52% in the two highest dose groups) and circulating CD4+ count. Other lymphocyte subpopulations showed no major changes.[20] This therapy demonstrated to be well tolerated and no opportunistic infections occurred. No further controlled trials have been performed, because of the resulting CD4 depletion that in some patients sustained for more than 12 months following treatment. Ongoing research also revealed that only subsets of activated T cells within the CD4+ compartment are important in the pathogenesis of inflammation in Crohn's disease.[7] A reduction of *all* CD4+ T cells would no longer seem to be a primary goal of immunotherapy.

Secondly, antibodies that neutralise α4β7 integrin have been proposed as another way to interfere with the T-cell activation in Crohn's disease. The α4β7 'gut homing integrin', a molecule important for selectively directing lymphocytes to the mucosal compartment, mediates adherence of lymphocytes to the activated cells of high endothelial venules (HEV's) or Payer's patches that express MadCAM-1. It is responsible for the specific recirculation of T cells through the intestinal mucosa.[21,22,23] Indeed, in cottontop tamarins, α4β7 neutralising antibodies reduced the severity of a spontaneously occurring colitis.[24]

Cytokine-targeting Therapies

In recent years TNF-α has been identified as a major pro-inflammatory cytokine in Crohn's disease.[18,25] TNF-α is a 17 kD non-glycosylated cytokine mainly produced by monocytes, macrophages and activated T-cells. After release, which is a result of clipping of the signal peptide by a specific metalloproteinase, TNF-α is released as a bioactive 51 kD trimer. Unclipped TNF-α remains membrane bound and is also biologically active upon contact with cells, that express a receptor for TNF-α (TNFR-1 or TNFR-2).[26] Of relevance for IBD are the abilities of TNF-α to recruit circulating inflammatory cells to local tissue sites of inflammation, to induce oedema, to activate coagulation activation, an its pivotal role in granuloma formation. Both in mice models and in humans, colitis is characterised by mucosal expression of high levels of TNF-α and IFNγ mRNA. The severity of TNBS-induced colitis could be reduced by (repeated) administration of neutralising anti-TNF-α or anti-IFNγ antibodies. Conversely, in TNF-α deficient mice colitis could not be induced by TNBS administration.[15] The chimeric monoclonal antibody infliximab, also known as cA2, has been studied in more patients with

steroid refractory Crohn's disease and/or fistulae.[27] This genetically constructed IgG1 murine-human chimeric monoclonal antibody binds to both the soluble subunit and membrane bound precursor of human TNF-α.[28]

In the first controlled clinical trial, 108 patients with moderate to severe Crohn's disease resistant to standard therapy, received placebo or cA2 at a dose of either 5, 10, 20 mg/kg.[29] The primary endpoint was a clinical response as defined by a decrease of the CDAI by more than 70 points, 4 weeks following administration of the antibody. The placebo response rate was 17%, versus 81% of the patients given 5 mg/kg of cA2, 50% in the 10 mg/kg group, and 64% in the 20 mg/kg group. Administration of the antibody as a single infusion resulted in remissions that were maintained in almost all patients that had responded to initial treatment during the 3-month study period. Although no important short-term side effects were encountered, the long-term effects of chronic or intermittent use remain unknown.

Preliminary results of a controlled study to evaluate the efficacy and safety of cA2 for the closure of enterocutaneous fistulae in Crohn's disease showed an impressive reduction in the number of draining fistulae (publication in preparation).

In another study, 31 patients with active Crohn's disease received a single infusion of a different anti-TNF-α antibody (the humanised antibody CDP571). Disease activity was reduced in the CDP571-treated patients: the CDAI dropped after two weeks from 263 to 167, in the placebo group no difference was observed.[30] In an open label trial 15 ulcerative colitis patients showed consistent improvement in disease activity in the initial 2 weeks after a single infusion of CDP571 and the treatment was well tolerated.[29]

The mechanism of action of anti-TNF-α antibodies remains to be revealed. Neutralisation of released TNF-α or membrane-bound TNF-α may be involved.[25,28]

In conclusion, anti-TNF-α treatment may induce clinical responses in patients with (steroid refractory) Crohn's disease and possibly also in ulcerative colitis. The induction of remission occurs rapidly, and is associated with a significant reduction of intestinal inflammation. Large phase III controlled clinical trials soon will start to study if maintenance of remission can be obtained with repeated infusions.

Alternative ways of interfering with production or release TNF-α are under investigation. These include TNF-α binding proteins, which have been constructed by placing the TNF-α binding domains of either TNFR-1 or TNFR-2 on an immunoglobulin backbone. In a large controlled trial with 185 rheumatoid arthritis patients, one of these proteins (recombinant human tumour necrosis factor receptor (p75)-Fc fusion protein) proved to be safe, well tolerated, and associated with improvement in the inflammatory symptoms.[32] Another approach is to increase the intracellular cyclic AMP concentrations thereby decreasing the TNF-α transcription. However using oxpentifylline, this approach showed no clinical efficacy in Crohn's disease.[33] Several metalloproteinase inhibitors can reduce TNF-α production in vitro and in vivo, and some are in clinical development. By the inhibition of clipping TNF-α, the release of TNF-α is blocked and the membrane bound TNF-α remains unaffected or may even accumulate. Clinical trials will have to answer the question whether released or membrane bound TNF-α is more important in the process of inflammation.[34]

Another candidate to restore the delicate balance between proinflammatory and anti-inflammatory cytokines in the intestinal mucosa is recombinant IL-10 (rIL-10). This is a 18 kD cytokine, produced by macrophages, monocytes and certain T and B cells. IL-10 is a potent inhibitor of activated macrophages and T cells by down regulation of IL-1, IL-6, IL-8 and TNF-α.[35] In addition, IL-10 interferes with antigen dependent T cell proliferation by reducing HLA class II expression.[36] Consequently, IL-10 favours Th2-type responses and B cell activation.[37] A phase II dose escalating study in 46 steroid-refractory patients with active Crohn's disease indicated the safety of a one week daily intravenous infusion of 0.5–25 g/kg rIL-10. The therapy was well tolerated and although the study was not designed to assess efficacy, 50% of the rIL-10 treated patients versus 23% of the placebo patients had a complete clinical remission.[38] However, preliminary data of a controlled trial investigating the efficacy of subcutaneous administration of rIL-10 in Crohn's disease patients showed less benefit (publication in preparation).

Conclusions

Experimental and clinical studies indicate that inhibition of specific inflammatory pathways may reduce severity of inflammatory bowel disease. Controlled clinical trials showed the potential benefit of anti-TNFα antibodies in Crohn's disease patients who were steroid-refractory or had enterocutaneous fistulae. Recombinant IL-10 seems less efficacious in decreasing activity of Crohn's disease when compared to anti-TNF-α. However, since IL-10 may inhibit a Th1–response it might prevent flare-ups or maintain remissions in Crohn's disease. Future studies will have to answer questions about the indications for use, benefit and toxicity of long term use and timepoint of administration. Disadvantages of cytokine-based therapies are possible induction of allergic reactions, antibody formation to the 'foreign' peptides that may lessen therapeutic effects, and increased susceptibility to opportunistic infections or malignancy (e.g. lymphoma's). Finally, these therapies are expected to be quite expensive.

A better understanding of the causative mechanisms underlying inflammatory bowel disease will result in more therapeutical strategies in Crohn's disease. These will include targeting cytokine gene transcription factors and cytokine-based gene therapy.[39] For example, the transient local expression of adenovirus-IL-4 in TNBS colitis in rats was shown to have a beneficial effect.[40] The main challenge for future management of Crohn's disease will be to develop disease-modifying interventions as well as strategies that effectively maintain disease remission.

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